

## FVB/N or C57BL/6 CRISPR injections

**Service:** Pronuclear stage zygotes are injected with CRISPR reagents for the purpose of creating knockouts or knockins. We have observed increased efficiency when investigators have purchased all their reagents (Cas9 enzyme, nickase, sgRNA and DNA). In 3 recent (October '15) knockin projects, utilizing Cas9 nickase and two sgRNA9(s) there were 5/65, 9/73 and 6/59 (founders/pups screened). The source of reagents were:

1. Recombinant Cas9 nickase: PNA bio Inc ([www.pnabio.com](http://www.pnabio.com)); catalog number CN01; 50ug/vial. The company also provides Cas9 enzyme (see website for cat #).
2. sgRNA(s): PNA Bio Inc ([www.pnabio.com](http://www.pnabio.com)); (sgRNA synthesis service; 50ug lyophilized). No catalog number.
3. Oligo DNA (knockins): IDT ([www.idtdna.com](http://www.idtdna.com)) as a 4 nmole Ultramer DNA oligo.
4. For large-fragment knockins, such as ires-cre or GFP fluorescent proteins, a DNA vector containing homologous fragments needs to be constructed and provided by investigators.

The reagents are injected in the following concentrations (manufacturer recommendation): Cas 9 (200ng/ul), sgRNA(s) (100ng/ul), DNA (20-60ng/ul). A mix of the reagents totaling 50ul is made and is used for one week. Reagents may be presented to the Core in a lyophilized form or reconstituted in culture grade water with enough aliquots of the Cas 9 and sgRNA to allow the lab to make at least 3 mixes.

**Guarantee:** 1 founder

**Fees: FVB injections:** BNORC member (inside or outside BIDMC) = \$2,000  
Non-BNORC member (inside BIDMC) = \$3,500  
Non-BNORC member (outside BIDMC) = \$5,250

**C57Bl/6 injections:** BNORC member (inside or outside BIDMC) = \$3,200  
Non-BNORC member (inside BIDMC) = \$5,000  
Non-BNORC member (outside BIDMC) = \$7,500

For More information contact:

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